

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
15 August 2002 (15.08.2002)

PCT

(10) International Publication Number
WO 02/062794 A2

(51) International Patent Classification⁷: **C07D 471/04**,
A61K 31/4375, A61P 1/16, 13/12 // (C07D 471/04,
211:00, 211:00)

(74) Agent: **ROWDEN, Janette, Yvonne**; Corporate Intel-
lectual Property, GlaxoSmithKline, 980 Great West Road
(CN925.1), Brentford, Middlesex TW8 9GS (GB).

(21) International Application Number: **PCT/EP02/00939**

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,
YU, ZA, ZM, ZW.

(22) International Filing Date: 30 January 2002 (30.01.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0102672.3 2 February 2001 (02.02.2001) GB

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG).

(71) Applicant (*for all designated States except US*): **GLAXO
GROUP LIMITED** [GB/GB]; Glaxo Wellcome House,
Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

(72) Inventors; and

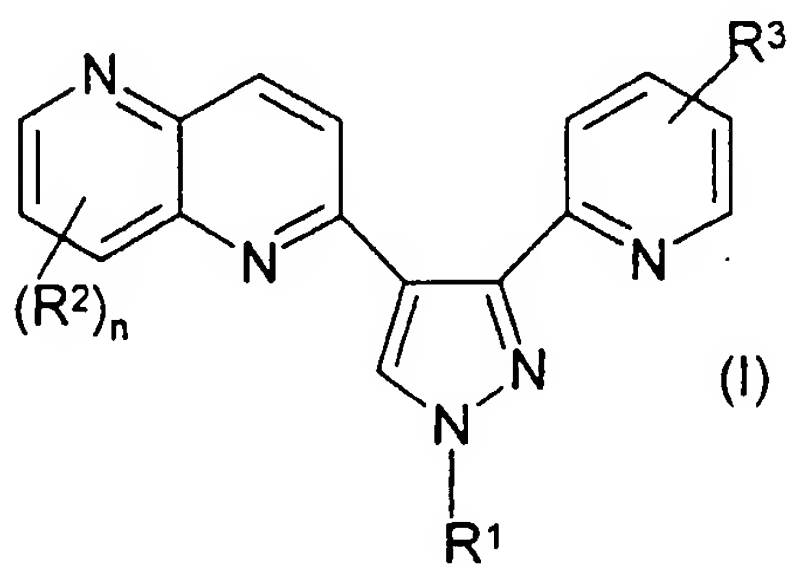
(75) Inventors/Applicants (*for US only*): **GELLIBERT,
Françoise, Jeanne** [FR/FR]; Laboratoire GlaxoSmithK-
line, Centre de Recherches, Z A de Courtabouef, 25
Avenue de Quebec, F-91940 Les Ulis (FR). **HARTLEY,
Charles, David** [GB/GB]; GlaxoSmithKline, Gunnels
Wood Road, Stevenage, Hertfordshire SG1 2NY (GB).
MATHEWS, Neil [GB/GB]; c/O GlaxoSmithKline, Gun-
nels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB).
WOOLVEN, James, Michael [GB/GB]; GlaxoSmithK-
line, Gunnels Wood Road, Stevenage, Hertfordshire SG1
2NY (GB).

Published:

— *without international search report and to be republished
upon receipt of that report*

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: COMPOUNDS



(57) Abstract: Therapeutically active pyrazole derivatives of formula (I) wherein R¹-R³ are as defined in the specification, processes for the preparation thereof, the use thereof in therapy, particularly in the treatment or prophylaxis of disorders characterised by overexpression of transforming growth factor β (TGF- β), and pharmaceutical compositions for use in such therapy.

COMPOUNDS

5 The present invention relates to novel pyrazole derivatives, processes for the preparation thereof, the use thereof in therapy, particularly in the treatment or prophylaxis of disorders characterised by overexpression of transforming growth factor β (TGF- β), and pharmaceutical compositions for use in such therapy.

10 TGF- β is a multi-functional cytokine which belongs to the TGF- β superfamily which includes activins/inhibins, bone morphogenetic proteins (BMPs) and TGF- β s. Three isoforms of TGF- β (TGF- β 1, TGF- β 2, and TGF- β 3) have been identified in mammals, each of which is encoded by a distinct gene on different chromosomes (D.A. Lawrence, *Eur. Cytokine. Netw.*, 1996, 7(3), 363). TGF- β initiates an intracellular signalling pathway which ultimately leads to the expression of genes that regulate cell cycle, control proliferative responses, or relate to extracellular matrix proteins that mediate cell
15 adhesion, migration and intercellular communication. TGF- β has pleiotropic effects including modulation of cell growth and differentiation, extracellular matrix formation, hematopoiesis, and immunomodulation (Roberts and Spoon, *Handbook of Experimental Pharmacology*, 1990, 95, 419-458).

20 A variety of cell surface proteins and receptors are known to transduce the signals initiated by the binding of the active TGF- β ligand to its receptors. Initiation of the TGF- β signalling pathway results from the binding of the TGF- β ligand to the extracellular domain of the type II membrane receptor (Massague, *Ann. Rev. Biochem.*, 1998, 67, 753.). The bound type II receptor then recruits type I (Alk5) receptor into a multimeric
25 membrane complex, whereupon active type II receptor kinase phosphorylates and activates type I receptor kinase. The function of the type I receptor kinase is to phosphorylate a receptor-associated co-transcription factor, Smad-2 or Smad-3; thereby releasing it into the cytoplasm where it binds to Smad-4. The PAI-1 gene is activated by TGF- β as a consequence of the abovementioned cellular pathway.

30 One approach to the treatment and/or prophylaxis of disorders characterised by the overexpression of TGF- β is inhibition of the TGF- β signal transduction. For example, inhibition of the TGF- β type II receptor by overexpression of a dominant negative TGF- β type II receptor has previously been shown to prevent liver fibrosis and dysfunction in

rat models (*Proc. Natl. Acad. Sci*, 1999, **96**(5), 2345), and also to prevent progression of established liver fibrosis (*Hepatology*, 2000, **32**, 247).

5 Pathological overexpression of TGF- β is known to be associated with a number of undesirable effects, leading ultimately to the development of serious pathogenic conditions (G.C. Blobe *et al.*, *N. Engl. J. Med.*, 2000, 1350). In particular, pathological overexpression of TGF- β may cause excessive accumulation of extracellular matrix (ECM), inhibition of cell proliferation and immunosuppression. Excessive accumulation of ECM is known to lead to fibrotic diseases such as tumor fibrosis, radiation-induced
10 fibrosis, fibrosis of the liver, kidney, lung, bowel, heart, pancreas, peritoneum or other organs. Fibrosis can lead to pathologic conditions such as cirrhosis, idiopathic pulmonary fibrosis, glomerulosclerosis and hypertrophic scars.

15 A number of other disease states are known to be associated with variations in the expression of genes which are controlled by TGF- β including cancer development, abnormal bone function and inflammatory disorders.

The development of compounds capable of inhibiting the TGF- β intracellular pathway is seen as a desirable way to effect prophylaxis and/or treatment of the above-mentioned
20 conditions. Compounds capable of inhibiting the TGF- β intracellular pathway and/or the expression of TGF- β may be used in the treatment of disorders the symptoms of which often lead to the development of fibrotic conditions. For example, compounds of the present invention may be useful in treating the fibrosis associated with various liver-related conditions such as hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol-induced hepatitis, haemochromatosis and primary biliary cirrhosis.
25

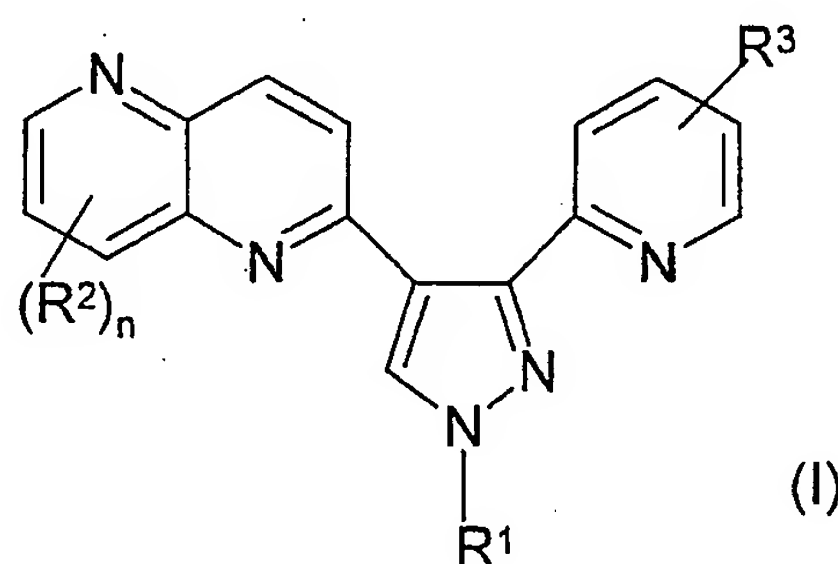
The compounds of the present invention are pyrazole derivatives. Other pyrazole compounds have previously been described for use in alternative medicinal applications. PCT Patent Applications , WO 96/03385, WO 98/52937, WO 98/52940,
30 WO 98/52941 and WO 00/31063 (Searle & Co) disclose a series of substituted pyrazole compounds and their use in the treatment of p38 kinase mediated disorders. In particular the compounds described are useful in the treatment of inflammation, inflammatory bowel disease, multiple sclerosis and asthma. European Patent Application No. 0 846 687 (Lilly & Co) describes novel substituted pyrazoles useful for
35 the inhibition of sPLA₂ mediated release of fatty acids. Such compounds are useful in

the treatment of conditions such as septic shock. EP 0 846 686 (Pfizer Ltd) discloses a series of condensed pyrazole derivatives which act as inhibitors of both Interleukin-1 (IL-1) and tumor necrosis factor (TNF). Such compounds are useful in the treatment of IL-1 and TNF mediated diseases such as chronic inflammatory diseases, specific autoimmune disease and sepsis-induced organ injury. None of the aforementioned patent applications describe the pyrazole compounds of the present invention.

PCT Patent Application WO 00/12947 (Scios Inc.) describes the use of a series of quinazoline derivatives for treating various disorders associated with enhanced activity of kinase p38- α and/or TGF- β . The compounds described therein have been shown to inhibit the activities of both proteins and are therefore particularly useful for the treatment of conditions in which an enhanced activity towards both p38- α and TGF- β is required.

It has now been discovered that certain substituted pyrazole compounds, as described below, are useful in the treatment or prophylaxis of disorders characterised by the overexpression of TGF- β . In particular, compounds of the present invention are TGF- β inhibitors which act at the TGF- β type I (Alk5) receptor level.

According to one aspect of the present invention, we provide compounds of formula (I),



wherein,

R^1 is selected from H, C_{1-4} alkyl or $CH_2CONR^4R^5$, wherein R^4 is selected from H or C_{1-4} alkyl and R^5 is C_{1-4} alkyl;

n is an integer selected from 0, 1, 2, 3, 4, or 5;

R^2 , which may be the same or different, is selected from halo (such as fluoro, chloro, bromo), -CN, -CF₃, -OCF₃, C₁₋₄ alkyl or C₁₋₄ alkoxy; and

R^3 is selected from H, halo (such as fluoro, chloro, bromo), -CN, -CF₃, C₁₋₄ alkyl or C₁₋₄ alkoxy;

and salts and solvates thereof (hereinafter "compounds of the invention").

The present invention also covers the physiologically acceptable salts of the compounds of formula (I). Suitable physiologically acceptable salts of the compounds of formula (I) include acid salts, for example sodium, potassium, calcium, magnesium and tetraalkylammonium and the like, or mono- or di- basic salts with the appropriate acid for example organic carboxylic acids such as acetic, lactic, tartaric, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids and inorganic acids such as hydrochloric, sulfuric, phosphoric and sulfamic acids and the like.

The present invention also relates to solvates of the compounds of Formula (I), for example hydrates.

Compounds of formula (I) where R^1 is H may exist in tautomeric forms. The individual tautomers and mixtures thereof are included within the scope of the present invention.

Preferably, R^1 is H or C₁₋₄ alkyl, more preferably R^1 is H.

Preferably, n is 0, 1 or 2, and R^2 is located at the C(2), C(3) or C(4) position of the naphthyridine ring and is selected from halo (such as fluoro, chloro, bromo), -CN, -CF₃, -OCF₃, C₁₋₄ alkyl or C₁₋₄ alkoxy. Most preferably, n is 0.

Preferably, R^3 is located at the C(3) or C(6) position of the pyridine ring and is selected from H, halo (such as fluoro, chloro, bromo), -CN, -CF₃, C₁₋₄ alkyl or C₁₋₄ alkoxy. More preferably R^3 is H or C₁₋₄ alkyl. Alternatively, R^3 is H.

It will be appreciated that the present invention is intended to include compounds having any combination of the preferred groups as listed hereinbefore.

- 5 Compounds of formula (I) which are of special interest as an agent useful in the treatment or prophylaxis of disorders characterised by the overexpression of TGF- β are: 2-(3-pyridin-2-yl-1*H*-pyrazol-4-yl)-[1,5]-naphthyridine; and 2-[3-(6-methylpyridin-2-yl)-1*H*-pyrazol-4-yl]-[1,5]-naphthyridine; and salts and solvates thereof.

10

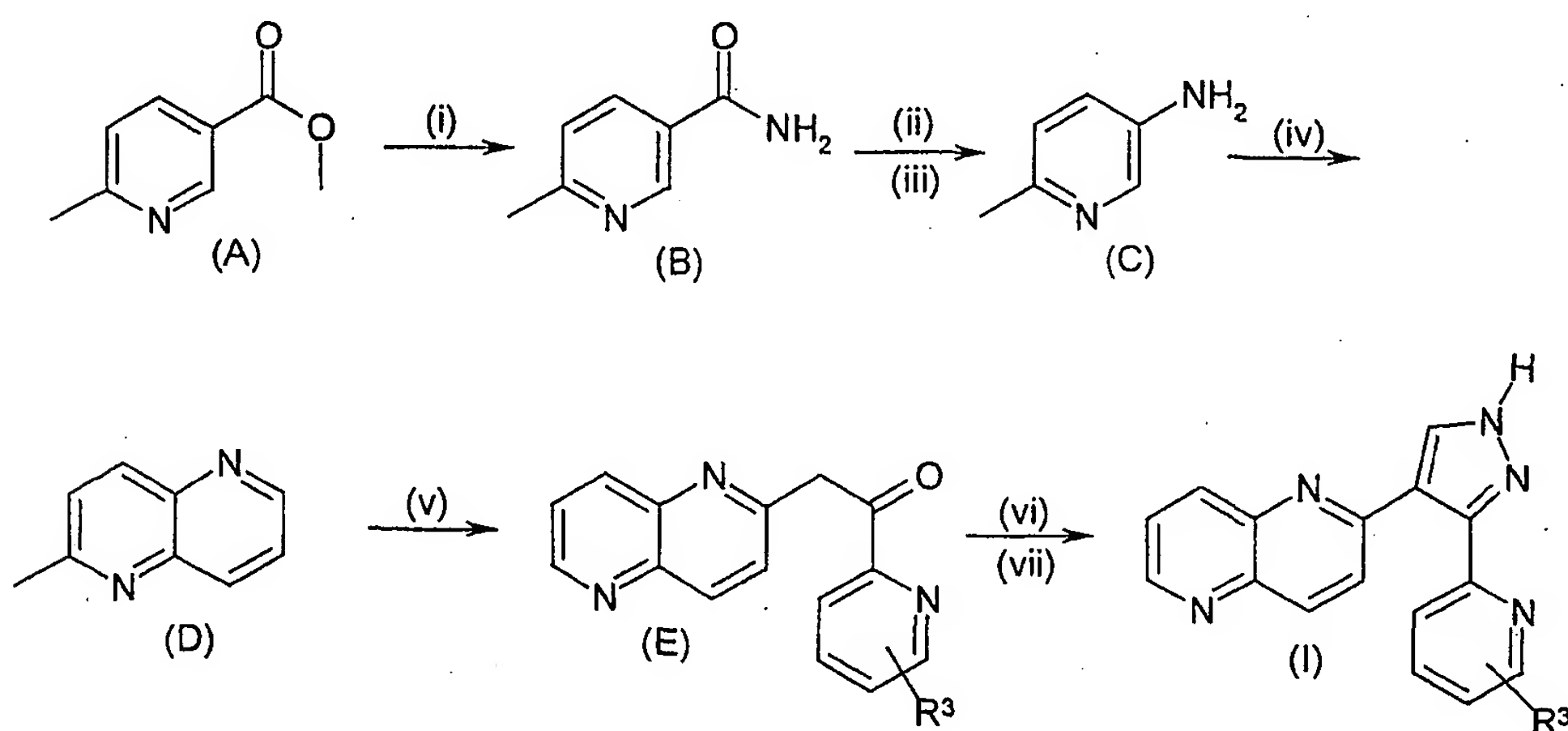
Compounds of formula (I) and salts and solvates thereof may be prepared by the methodology described hereinafter, constituting a further aspect of this invention. In yet a further aspect of the present invention there is provided a process for the preparation of intermediate compounds of formula (E).

15

Compounds of formula (I) may conveniently be prepared according to the general methodology in Scheme 1.

Scheme 1

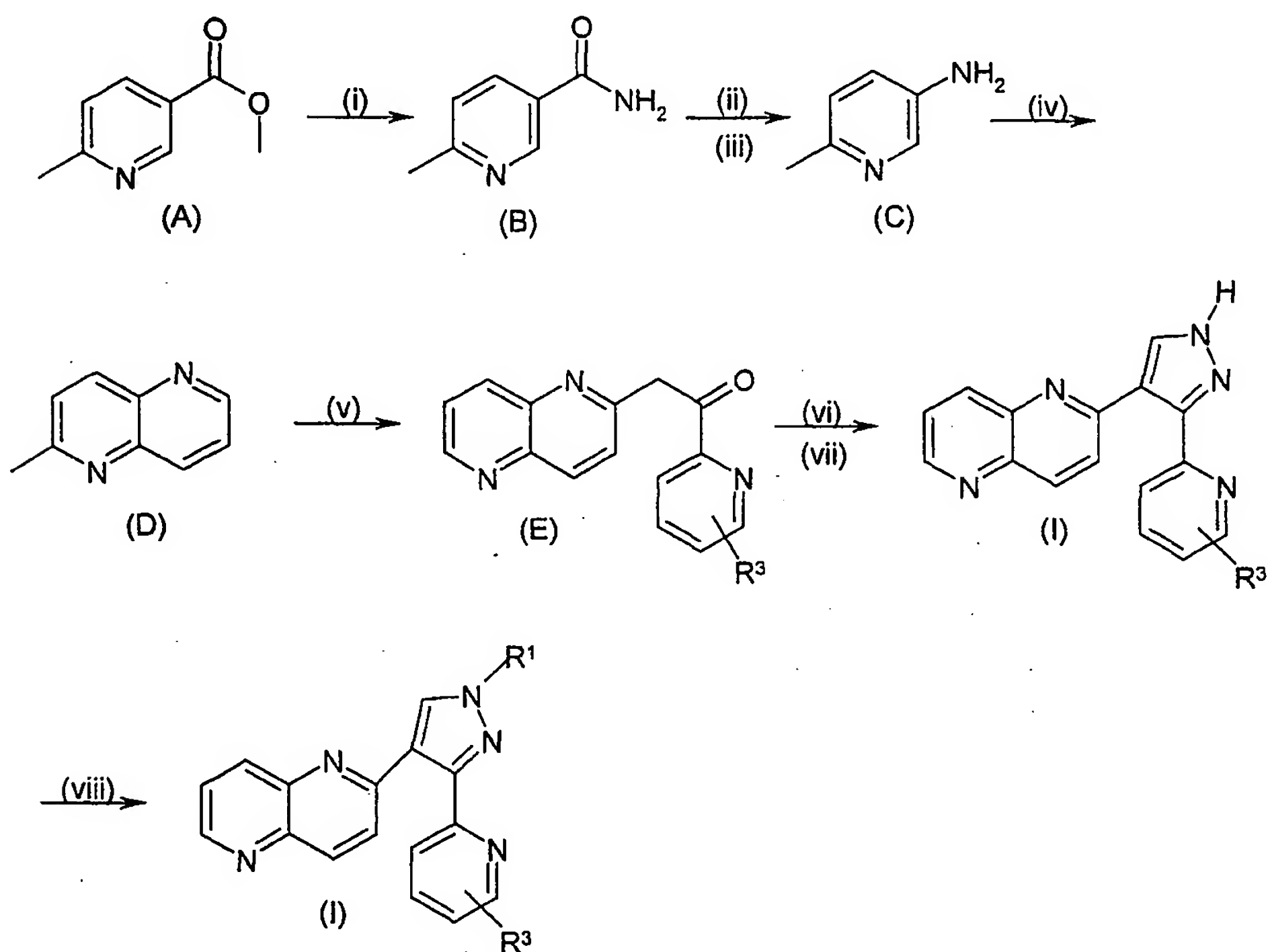
20



Reagents and conditions (preferred): (i) NH_4OH ; (ii) Br_2 , NaOH(aq) ; (iii) H_2SO_4 (conc), sodium m-nitrobenzenesulphonate, H_3BO_3 , $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; (iv) glycerol, H_2O ; (v) $\text{R}^3(\text{C}_5\text{H}_3\text{N})\text{CO}_2\text{Et}$, KHMDs , THF , -78°C ; (vi) AcOH , DMF.DMA , DMF , r.t.; (vii) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, DMF .

5

Scheme 2



10 Reagents and conditions (preferred): (i) NH_4OH , r.t.; (ii) Br_2 , NaOH(aq) ; (iii) H_2SO_4 (conc), sodium m-nitrobenzenesulphonate, H_3BO_3 , $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; (iv) glycerol, H_2O ; (v) $\text{R}^3(\text{C}_5\text{H}_3\text{N})\text{CO}_2\text{Et}$, KHMDs , THF , -78°C ; (vi) AcOH , DMF.DMA , DMF , r.t.; (vii) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, DMF ; (viii) R^1X , K_2CO_3 , DMF , r.t.

15 List of Abbreviations

AcOH	Acetic acid
DMF.DMA	Dimethylformamide dimethylacetal
DMF	Dimethylformamide

KHMDS	Potassium <i>bis</i> (trimethylsilyl)amide
MeCN	Acetonitrile
THF	Tetrahydrofuran

5 In a further aspect of the present invention, compounds of Formula (I) where R^1 is selected from C_{1-4} alkyl or $CH_2CONR^4R^5$, wherein R^4 and R^5 are hereinbefore defined, may be prepared by N-alkylation of a pyrazole compound of formula (I) obtained from step (vii) above, according to the following general procedure (Scheme 2):

- 10 (i) Addition of a suitable N-alkylating reagent such as an alkyl halide, R^1X (wherein R^1 is hereinbefore defined) in the presence of a suitable base such as potassium carbonate, sodium hydroxide or potassium hydroxide, preferably in the temperature range 0 to 75°C, more preferably in the temperature range 20 to 60°C, most preferably at room temperature, in the presence of a suitable solvent
15 such as DMF or MeCN, followed by separation of any resulting isomeric mixture by flash chromatography on silica gel.

It will be appreciated that N-alkylation according to step (viii) in Scheme 2 above may result in the formation of structural isomers of a compound of formula (I). Such isomers
20 are afforded by N-alkylation of the tautomeric forms of a compound of formula (I) where R^1 is H, mentioned hereinbefore. The individual isomers and mixtures thereof are included within the scope of the present invention.

25 6-Methyl-3-aminopyridine (C) may be prepared according to processes known in the art, for example, A.W. Hofmann, *Ber. Dtsch. Ges.*, 1881, **14**, 2725.

2-Methyl-1,5-naphthyridine (D) may be prepared according to processes known in the art, for example, *Chem. Pharm. Bull.*, 1971, **19(9)**, 1857.

30 Monosubstituted pyridyl esters, $R^3(C_5H_3N)CO_2Et$ (where R^3 is hereinbefore defined) as described in step (ii) above may be prepared by processes analogous to those known in the art. For example, where $R^3 = C(6)-OMe$, Finger *et al.*, *J. Org. Chem.*, 1962, **27**, 3965; where $R^3 = C(3)-OMe$, Dejardin *et al.*, *Bull. Chim. Soc. Fr.*, 1979, 289; where $R^3 = C(5)-Br$, Chambers and Marfat, *Synth. Commun.*, 1997, **27(3)**, 515; and where $R^3 = C(4)-CN$, Heinisch and Lotsch, *Heterocycles*, 1987, **26(3)**, 731.
35

N-alkylation reactions as described in step (v) may be performed according to processes analogous to those known in the art (e.g. R. Fusco, Pyrazoles, Chapter 4, p.71, *The Chemistry of Heterocyclic Compounds*, A. Weissberger (ed), Vol. 22, Intersciences, New York, 1967 and Elguero, Pyrazoles and their Benzo derivatives, p.222, *Comprehensive Heterocycles Chemistry*, A.R. Katrisky, C.W. Rees and K.T. Potts (eds), Vol. 5, Pergamon Press, Oxford, 1984).

The compounds of the present invention have been found to inhibit phosphorylation of the Smad-2 or Smad-3 proteins by inhibition of the TGF- β type 1 (Alk5) receptor.

Accordingly, the compounds of the invention have been tested in the assays described herein and have been found to be of potential therapeutic benefit in the treatment and prophylaxis of disorders characterised by the overexpression of TGF- β .

Thus there is provided a compound of formula (I) or a physiologically acceptable salt or solvate thereof for use as a medicament in human or veterinary medicine, particularly in the treatment or prophylaxis of disorders characterised by the overexpression of TGF- β .

It will be appreciated that references herein to treatment extend to prophylaxis as well as the treatment of established conditions. It will further be appreciated that references herein to treatment or prophylaxis of disorders characterised by the overexpression of TGF- β , shall include the treatment or prophylaxis of TGF- β associated disease such as fibrosis, especially liver and kidney fibrosis, cancer development, abnormal bone function, and inflammatory disorders, or scarring.

Other pathological conditions which may be treated in accordance with the invention have been discussed in the introduction hereinbefore. The compounds of the present invention are particularly suited to the treatment of fibrosis and related conditions.

Compounds of the present invention may be administered in combination with other therapeutic agents, for example antiviral agents for liver diseases or in combination with ACE inhibitors or Angiotensin II receptor antagonists for kidney diseases.

According to another aspect of the invention, there is provided the use of a compound of formula (I) or a physiologically acceptable salt or solvate thereof for the manufacture of a medicament for the treatment and/or prophylaxis of disorders characterised by the overexpression of TGF- β , particularly fibrosis.

5

In a further aspect there is provided a method for the treatment of a human or animal subject with a disorder characterised by the overexpression of TGF- β , particularly fibrosis, which method comprises administering to said human or animal subject an effective amount of a compound of formula (I) or a physiologically acceptable salt or solvate thereof.

10

Compounds of the invention may be formulated for administration in any convenient way, and the invention therefore also includes within its scope pharmaceutical compositions for use in therapy, comprising a compound of formula (I) or a physiologically acceptable salt or solvate thereof in admixture with one or more physiologically acceptable diluents or carriers.

15

There is also provided according to the invention a process for preparation of such a pharmaceutical composition which comprises mixing the ingredients.

20

Compounds of the invention may, for example, be formulated for oral, buccal, parenteral, topical or rectal administration.

25

Tablets and capsules for oral administration may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, mucilage of starch or polyvinyl pyrrolidone; fillers, for example, lactose, microcrystalline cellulose, sugar, maize- starch, calcium phosphate or sorbitol; lubricants, for example, magnesium stearate, stearic acid, talc, polyethylene glycol or silica; disintegrants, for example, potato starch, croscarmellose sodium or sodium starch glycollate; or wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin,

30

35

hydroxymethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example, lecithin, sorbitan mono-oleate or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters, propylene glycol or ethyl alcohol; or
5 preservatives, for example, methyl or propyl *p*-hydroxybenzoates or sorbic acid. The preparations may also contain buffer salts, flavouring, colouring and/or sweetening agents (e.g. mannitol) as appropriate.

For buccal administration the compositions may take the form of tablets or lozenges
10 formulated in conventional manner.

The compounds may also be formulated as suppositories, e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

15 Compounds of the invention may also be formulated for parenteral administration by bolus injection or continuous infusion and may be presented in unit dose form, for instance as ampoules, vials, small volume infusions or pre-filled syringes, or in multi-dose containers with an added preservative. The compositions may take such forms as solutions, suspensions, or emulsions in aqueous or non-aqueous vehicles, and may
20 contain formulatory agents such as anti-oxidants, buffers, antimicrobial agents and/or toxicity adjusting agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use. The dry solid presentation may be prepared by filling a sterile powder aseptically into individual sterile containers or by filling a sterile solution aseptically into each container and
25 freeze-drying.

By topical administration as used herein, we include administration by insufflation and inhalation. Examples of various types of preparation for topical administration include ointments, creams, lotions, powders, pessaries, sprays, aerosols, capsules or
30 cartridges for use in an inhaler or insufflator or drops (e.g. eye or nose drops).

Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents and/or solvents. Such bases may thus, for example, include water and/or an oil such as liquid paraffin or a
35 vegetable oil such as arachis oil or castor oil or a solvent such as a polyethylene glycol.

Thickening agents which may be used include soft paraffin, aluminium stearate, cetostearyl alcohol, polyethylene glycols, microcrystalline wax and beeswax.

5 Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents or thickening agents.

10 Powders for external application may be formed with the aid of any suitable powder base, for example, talc, lactose or starch. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilising agents or suspending agents.

15 Spray compositions may be formulated, for example, as aqueous solutions or suspensions or as aerosols delivered from pressurised packs, with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, 1,1,1,2,3,3,3-heptafluoropropane, 1,1,1,2-tetrafluoroethane, carbon dioxide or other suitable gas.

20 Capsules and cartridges for use in an inhaler or insufflator, of for example gelatin, may be formulated containing a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

25 Compounds of the invention may conveniently be administered in amounts of, for example, 0.01 to 100mg/kg body weight, suitably 0.05 to 25mg/kg body weight orally, one or more times a day. The precise dose will of course depend on the age and condition of the patient, the particular route of administration chosen, and is entirely within the discretion of the administering physician.

30 The following non-limiting Examples illustrate the present invention.

Intermediates

Intermediate 1: 2-Methyl-[1,5]naphthyridine

35 A mixture of concentrated sulphuric acid (14 ml), sodium m-nitrobenzenesulphonate (11.30 g), boric acid (1.55 g, 0.039 mol) and iron sulphate heptahydrate (0.90 g, 3.23

mmol) was stirred at room temperature. Glycerol (8.0 ml) was added followed by 3-amino-6-methyl-pyridine (2.79 g, 0.025 mol) and water (14 ml). The resultant mixture was heated at 135 °C with stirring for 18 h. The reaction mixture was allowed to cool to room temperature, basified using 4N sodium hydroxide and the resultant mixture was extracted using ethyl acetate (X4). The extracts were combined and then preadsorbed onto silica gel (20 ml) prior to Biotage chromatography (using a 90 g silica gel cartridge) and eluting with ethyl acetate (neat). Appropriate fractions were combined and then evaporated to give the title compound (2.01 g, 55%) as a light brown crystalline solid.

LC-MS m/z 145 = MH+

Intermediate 2: 2-[1,5]Naphthyridin-2-yl-1-pyridin-2-yl-ethanone

To a stirred and cooled (-78 °C) solution of 2-methyl-[1,5]naphthyridine, (0.50 g, 3.46 mmol) and ethyl picolinate (0.52 g, 3.47 mmol) in anhydrous THF (30 ml) was added potassium hexamethyldimethylsilazide (0.5M solution in toluene) (13.9 ml, 6.94 mmol) dropwise over 10 minutes. This mixture was stirred at -78 °C for 1 h and then at room temperature for 20 h. Saturated aqueous ammonium chloride (100 ml) was added to the reaction mixture with stirring and the resultant mixture was partitioned between ethyl acetate and water. The aqueous phase was separated off and was extracted with ethyl acetate (X3). The extracts and organic phase were combined, washed with water and finally dried and evaporated to give the title compound (0.86 g) as an orange yellow solid.

[APCI MS] m/z 250 (MH+)

Intermediate 3: 1-(6-Methyl-pyridin-2-yl)- 2-[1,5]naphthyridin-2-yl-ethanone

2-Methyl-[1,5]naphthyridine (4.34g, 30.1mmol) and methyl-6-methyl picolinate (1.1eq, 5g, 33.11mmol) were coupled as described for intermediate 2 to afford the title compound as an orange solid (6g) .

[APCI MS] m/z 264 (MH+)

Examples

Example 1: 2-(3-Pyridin-2-yl-1H-pyrazol-4-yl)-1,5-naphthyridine

To a stirred solution of 2-[1,5]naphthyridin-2-yl-1-pyridin-2-yl-ethanone, (0.39 g, 1.56 mmol) in anhydrous DMF (6 ml) was added acetic acid (0.32 ml, 5.61 mmol) followed by dimethylformamide/dimethylacetamide (DMF/DMA) (0.62 ml, 4.68 mmol). This mixture was stirred at room temperature for 1 h and then hydrazine hydrate (1.70 ml, 35.0 mmol) was added. The resultant mixture was heated at 50 °C for 2 h and then allowed to cool to room temperature. The reaction was partitioned between water and ethyl acetate and then the aqueous phase was separated off and was extracted with ethyl acetate (X8). The extracts and organic phase were combined, dried (Na₂SO₄) and evaporated to give crude product as a red-brown gum. This gum was crystallised from acetonitrile to give the title compound (0.31 g, 73%) as a buff coloured solid.

[APCI MS] m/z 274 (MH⁺)

¹H NMR: (DMSO-d₆): δ 13.6 (1H, V.br.s, NH), 8.92 (1H, dd, CH), 8.58 (1H, br.s, CH), 8.33-8.25 (3H, br.m, 3 X CH), 7.95-7.80 (3H, 2 X m, 3 X CH), 7.74 (1H, dd, CH), 7.40 (1H, m, CH)

Example 2: 2-[3-(6-Methyl-pyridin-2-yl-1H-pyrazol-4-yl)-[1,5]-naphthyridine

Intermediate 3 (5.66g, 21.52mmol) was reacted with DMF/DMA (3.85g, 32.28mmol) and hydrazine hydrate (7.54g, 150mmol) to afford, after trituration in pentane, to the title compound as a yellow solid (2.06g).

m.p: 193°C.

HRMS calcd.Mass for C₁₇H₁₃N₅ : (M+H)⁺ 288.1249, found : 288.1228.

It will be appreciated that a degree of spectral line width broadening is inherent to the ¹H NMR spectra of these types of pyrazole compounds owing to the presence of different tautomeric forms and/or hindered rotation(s) within the molecules.

Biological Data

The compounds of Examples 1 and 2 were tested *in vitro*, using the biological Assay 1 described below. Both of the compounds tested had an IC₅₀ value of about 0.05 μM in Assay 1.

Assays

Assay 1

5 The potential for compounds of the invention to inhibit TGF- β signaling may be demonstrated, for example, using the following *in vitro* assay.

The assay was performed in HepG2 cells stably transfected with the PAI-1 promoter (known to be a strong TGF- β responsive promoter) linked to a luciferase (firefly) reporter gene. The compounds were selected on their ability to inhibit luciferase activity in cells exposed to TGF- β . In addition cells were transfected with a second luciferase (Renilla)
10 gene which was not driven by a TGF- β responsive promoter and was used as a toxicity control.

(96 well-)microplates are seeded, using a multidrop apparatus, with the stably transfected cell line at a concentration of 35000 cells per well in 200 μ l of serum-containing medium. These plates are placed in a cell incubator.

15 18 to 24 hours later (Day 2), cell-incubation procedure is launched. Cells are incubated with TGF- β and a candidate compound at concentrations in the range 50 nM to 10 μ M (final concentration of DMSO 1%). The final concentration of TGF- β (rhTGF β -1) used in the test is 1 ng/mL. Cells are incubated with a candidate compound 15-30 mins prior to the addition of TGF- β . The final volume of the test reaction is 150 μ l. Each well contains
20 only one candidate compound and its effect on the PAI-1 promoter is monitored.

Columns 11 and 12 are employed as controls. Column 11 contains 8 wells in which the cells are incubated in the presence of TGF- β , *without* a candidate compound. Column 11 is used to determine the 'reference TGF- β induced firefly luciferase value' against which values measured in the test wells (to quantify inhibitory activity) may be
25 compared. In wells A12 to D12, cells are grown in medium without TGF- β . The firefly luciferase values obtained from these positions are representative of the 'basal firefly luciferase activity'. In wells E12 to H12, cells are incubated in the presence of TGF- β and 500 μ M CPO (Cyclopentenone, Sigma), a cell toxic compound. The toxicity is

revealed by decreased firefly and renilla luciferase activities (around 50 % of those obtained in column 11).

12 to 18 hours later (day 3), the luciferase quantification procedure is launched. The following reactions are performed using reagents obtained from a Dual Luciferase Assay Kit (Promega). Cells are washed and lysed with the addition of 10 μ l of passive lysis buffer (Promega). Following agitation (15 to 30 mins), luciferase activities of the plates are read in a dual-injector luminometer (BMG lumistar). For this purpose, 50 μ l of luciferase assay reagent and 50 μ l of 'Stop & Glo' buffer are injected sequentially to quantify the activities of both luciferases. Data obtained from the measurements are processed and analysed using suitable software. The mean Luciferase activity value obtained in wells A11 to H11 (Column 11, TGF- β only) is considered to represent 100% and values obtained in wells A12 to D12 (cells in medium alone) give a basal level (0%). For each of the compounds tested, a concentration response curve is constructed from which an IC₅₀ value can be determined graphically.

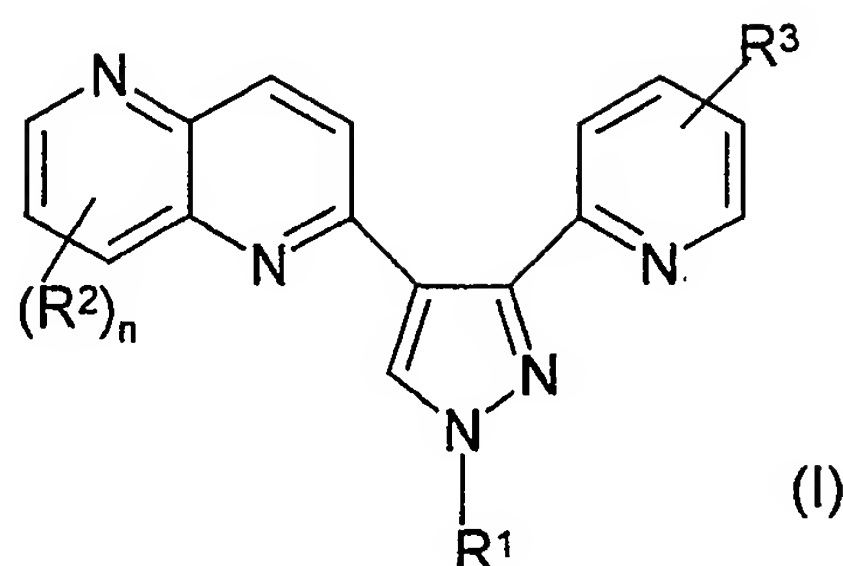
15 Assay 2

The potential for compounds of the invention to inhibit the kinase Alk5 receptor may be demonstrated, for example, using the following *in vitro* assay.

The kinase domain of Alk5 was cloned and expressed in a baculovirus/Sf9 cells system. The protein (amino acids 162 to 503) was 6-His tagged in C-terminus. After purification by affinity chromatography using a Ni²⁺ column, the autophosphorylation was tested. The enzyme was incubated in a medium containing : Tris 50 mM pH 7.4; NaCl 100 mM ; MgCl₂ 5 mM ; MnCl₂ 5 mM ; DTT 10 mM. The enzyme was preincubated with the compounds (0.1% DMSO final in the test) 10 minutes at 37°C. The reaction was initialised by the addition of 3 μ M ATP (0.5 μ Ci gamma-33P-ATP). After 15 minutes at 37°C the reaction was stopped by addition of SDS-PAGE sample buffer (50 mM Tris-HCl, pH 6.9, 2.5 % glycerol, 1% SDS, 5 % beta-mercaptoethanol). The samples were boiled for 5 minutes at 95°C and run on a 12% SDS-PAGE. The dried gels were exposed to a phosphor screen over-night. Alk5 autophosphorylation was quantified using a STORM (Molecular Dynamics).

Claims

- 5 1. A compound of formula (I),



wherein,

10

R^1 is selected from H, C_{1-4} alkyl or $CH_2CONR^4R^5$;

n is an integer selected from 0, 1, 2, 3, 4, or 5;

15

R^2 , which may be the same or different, is selected from halo (such as fluoro, chloro, bromo), -CN, -CF₃, -OCF₃, C_{1-4} alkyl or C_{1-4} alkoxy;

R^3 is selected from H, halo (such as fluoro, chloro, bromo), -CN, -CF₃, C_{1-4} alkyl or C_{1-4} alkoxy;

20

R^4 is selected from H or C_{1-4} alkyl and R^5 is C_{1-4} alkyl;

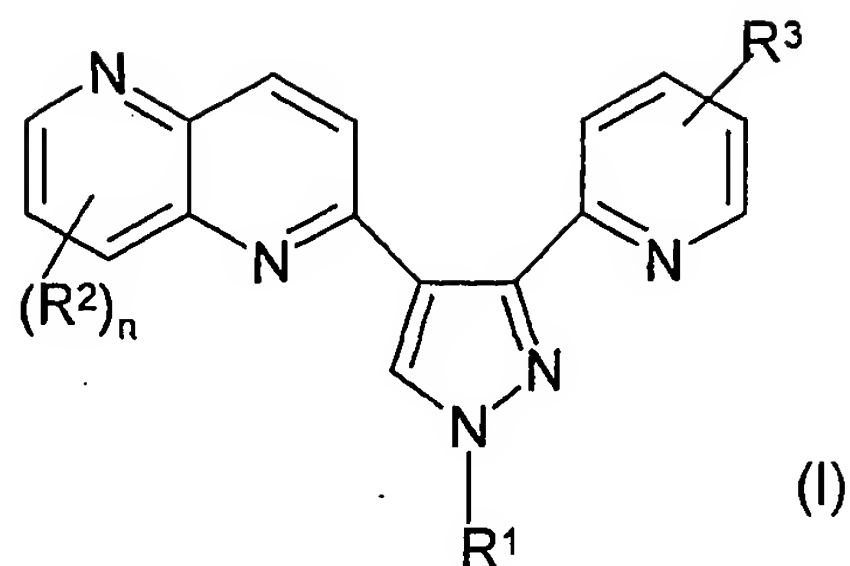
and salts and solvates thereof.

25

2. A compound of formula (I) as claimed in Claim 1 wherein R^1 is H or C_{1-4} alkyl.

3. A compound of formula (I) as claimed in Claim 1 or Claim 2, wherein n is 0,1 or 2 and R² is located at the C(2), C(3) or C(4) position of the naphthyridine ring and is selected from halo, -CN, -CF₃, -OCF₃, C₁₋₄ alkyl or C₁₋₄ alkoxy.
- 5 4. A compound of formula (I) as claimed in Claim 3, wherein n is 0.
5. A compound of formula (I) as claimed in any one of the preceding claims wherein R³ is located at the C(3) or C(6) position of the pyridine ring and is selected from H, halo, -CN, -CF₃, C₁₋₄ alkyl or C₁₋₄ alkoxy.
- 10 6. A compound of formula (I) as claimed in Claim 5, wherein R³ is H or C₁₋₄ alkyl.
7. A compound of formula (I) as claimed in Claim 1 selected from:
2-(3-pyridin-2-yl-1H-pyrazol-4-yl)-[1,5]naphthyridine; and
15 2-[3-(6-methylpyridin-2-yl-1H-pyrazol-4-yl)-[1,5]naphthyridine;
and salts and solvates thereof.
8. A pharmaceutical composition comprising at least one compound of formula (I) as claimed in any one of claims 1 to 7, together with a pharmaceutically acceptable diluent or carrier.
- 20 9. A compound of formula (I) as claimed in any one of claims 1 to 7, for use as a medicament.
- 25 10. The use of a compound of formula (I) as claimed in any one of claims 1 to 7 in the manufacture of a medicament for the treatment and/or prophylaxis of disorders characterised by the overexpression of TGF- β .
- 30 11. A method for the treatment of a human or animal subject with a disorder characterised by the overexpression of TGF- β , which method comprises administering to said human or animal subject an effective amount of a compound of formula (I) as claimed in any one of Claims 1 to 7 or a physiologically acceptable salt or solvate thereof.
- 35 12. A process for the preparation of a compound of formula (I),

18

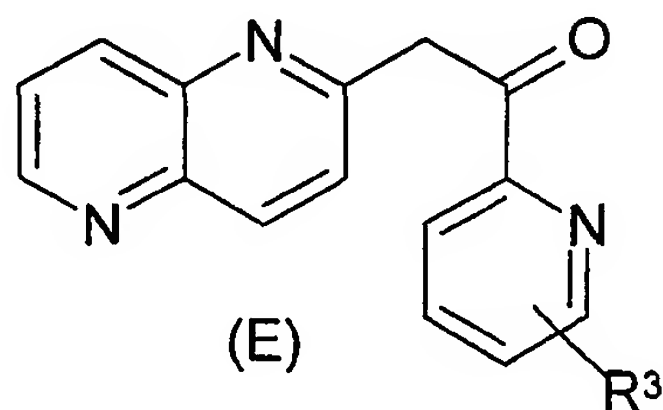


wherein,

- 5 R^1 is H; n is an integer selected from 0, 1, 2, 3, 4, or 5; R^2 , which may be the same or different, is selected from halo (such as fluoro, chloro, bromo), -CN, -CF₃, -OCF₃, C₁₋₄ alkyl or C₁₋₄ alkoxy; and R^3 is selected from H, halo (such as fluoro, chloro, bromo), -CN, -CF₃, C₁₋₄ alkyl or C₁₋₄ alkoxy;

- 10 which process comprises:

- a) addition of acetic acid followed by dimethylformamide dimethylacetal to a ketone of formula (E)



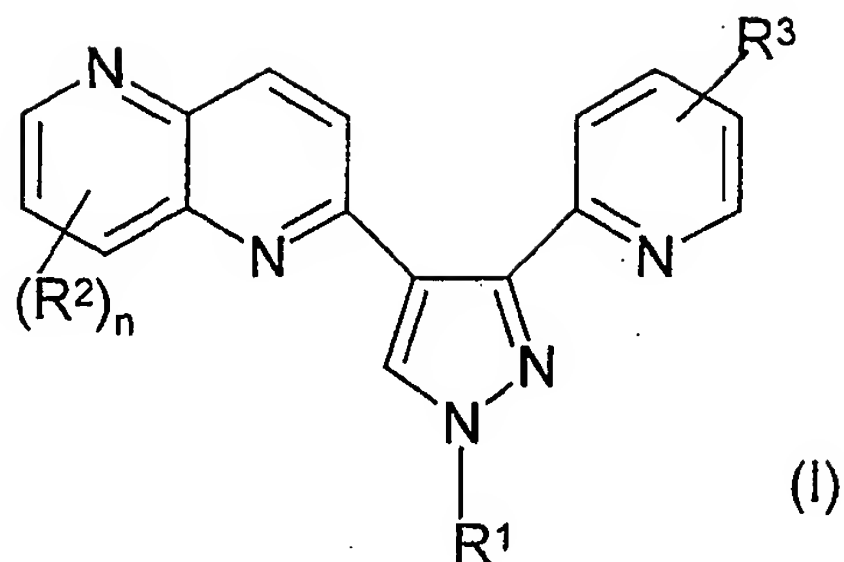
15

wherein R^3 is hereinbefore defined above; and

- 20 b) subsequent addition of hydrazine monohydrate, NH₂NH₂.H₂O to the resulting reaction mixture.

13. A process for the preparation of a compound of formula (I),

19



wherein,

- 5 R^1 is C_{1-4} alkyl or $CH_2CONR^4R^5$, wherein R^4 is selected from H or C_{1-4} alkyl and R^5 is C_{1-4} alkyl; n is an integer selected from 0, 1, 2, 3, 4, or 5; R^2 , which may be the same or different, is selected from halo, -CN, -CF₃, -OCF₃, C_{1-4} alkyl or C_{1-4} alkoxy; and R^3 is selected from H, halo, -CN, -CF₃, C_{1-4} alkyl or C_{1-4} alkoxy;

- 10 which process comprises N-alkylation of a compound of formula (I) where R^1 is H, in the presence of a suitable base.

15

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
15 August 2002 (15.08.2002)

PCT

(10) International Publication Number
WO 02/062794 A3

(51) International Patent Classification⁷: C07D 471/04, A61K 31/4375, A61P 1/16, 13/12 // (C07D 471/04, 221:00, 221:00)

(74) Agent: ROWDEN, Janette, Yvonne; Corporate Intellectual Property, GlaxoSmithKline, 980 Great West Road (CN925.1), Brentford, Middlesex TW8 9GS (GB).

(21) International Application Number: PCT/EP02/00939

(22) International Filing Date: 30 January 2002 (30.01.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0102672.3 2 February 2001 (02.02.2001) GB

(71) Applicant (for all designated States except US): **GLAXO GROUP LIMITED** [GB/GB]; Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **GELLIBERT, Françoise, Jeanne** [FR/FR]; Laboratoire GlaxoSmithKline, Centre de Recherches, Z A de Courtabouef, 25 Avenue de Quebec, F-91940 Les Ulis (FR). **HARTLEY, Charles, David** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB). **MATHEWS, Neil** [GB/GB]; c/o GlaxoSmithKline, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB). **WOOLVEN, James, Michael** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

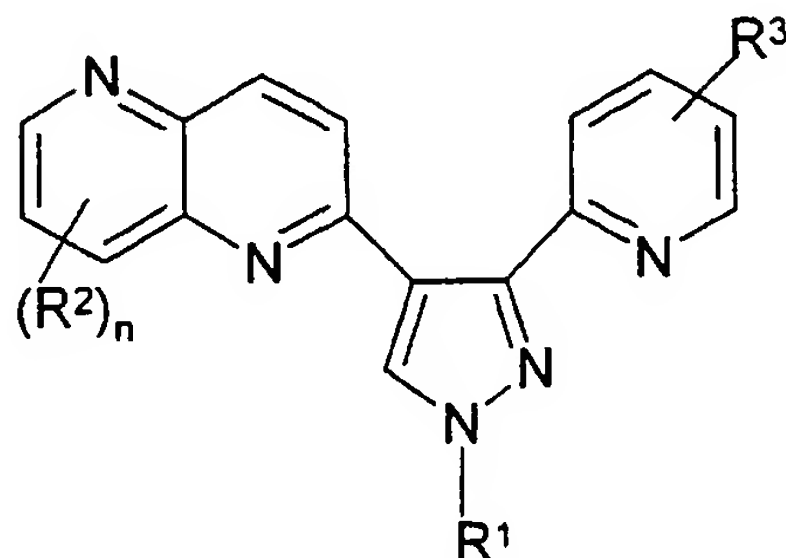
Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
3 October 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COMPOUNDS



(I)

(57) Abstract: Therapeutically active pyrazole derivatives of formula (I) wherein R¹-R³ are as defined in the specification, processes for the preparation thereof, the use thereof in therapy, particularly in the treatment or prophylaxis of disorders characterised by overexpression of transforming growth factor β (TGF- β), and pharmaceutical compositions for use in such therapy.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/00939

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D471/04 A61K31/4375 A61P1/16 A61P13/12
 //(C07D471/04,221:00,221:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 61576 A (SMITHKLINE BEECHAM) 19 October 2000 (2000-10-19) claims 1,10,11 ---	1,8,10
A	WO 00 69847 A (ORTHO MCNEIL) 23 November 2000 (2000-11-23) claims 1,22 -----	1,8,10

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *8* document member of the same patent family

Date of the actual completion of the international search

13 August 2002

Date of mailing of the international search report

21/08/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Alfaro Faus, I

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 02/00939

Box I Observation where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 11 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 02/00939

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 0061576	A	19-10-2000	AU	4798000 A	14-11-2000
			EP	1169317 A1	09-01-2002
			WO	0061576 A1	19-10-2000
<hr/>					
WO 0069847	A	23-11-2000	AU	4677700 A	05-12-2000
			BR	0010560 A	04-06-2002
			CN	1361773 T	31-07-2002
			EP	1178981 A1	13-02-2002
			WO	0069847 A1	23-11-2000
<hr/>					